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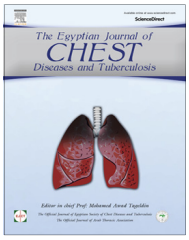

ORIGINAL ARTICLE

C-reactive protein and serum amyloid A levels in discriminating malignant from non-malignant pleural effusion

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KEYWORDS

Pleural effusion;
C-reactive protein;
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Abstract *Introduction:* Distinction between malignant and non-malignant pleural effusion is of great importance in the patient management.

The aim: We examined the diagnostic value of C-reactive protein (CRP) and serum amyloid A (SAA) in distinguishing different etiologies of pleural effusion and if they could discriminate between malignant and non-malignant pleural effusions.

Subject and methods: CRP and SAA levels in both serum and pleural fluid were measured in 92 patients with pleural effusion. Of the 92 patients included in our study; 44 were diagnosed with malignant pleural effusions (group I) [with male to female ratio (M/F) 23/21 and mean age 57.7 ± 11.5 years in the form of mean ± 2SD] and 48 were diagnosed with non-malignant pleural effusion (group II) [with M/F ratio 33/15 and mean age 54.7 ± 10.4 years in the form of mean ± 2SD].

Results: CRP and SAA values were significantly higher in both serum and pleural effusion of malignant vs. non-malignant group ($P < 0.003$), but there was no statistical significant difference as regards pleural/serum CRP and pleural/serum SAA ratios between the two groups ($P = 0.148$ and $P = 0.453$ respectively). A statistically significant positive correlation between pleural fluid CRP and pleural fluid SAA in malignant and non-malignant effusions was detected ($r = 0.315$ and $P = 0.002$ respectively). Diagnostic performance of pleural fluid CRP and pleural fluid SAA in both infectious and malignant pleural effusions showed that at a cutoff value of 96.15 μg/ml for CRP; diagnostic sensitivity was 61% and specificity was 45%, while for pleural fluid SAA, a cutoff value of 137.5 μg/ml was associated with 41% sensitivity and 93% specificity.

Conclusion: Measurement of SAA and CRP levels in pleural fluid has good diagnostic utility in differentiation between malignant and non-malignant pleural effusion and pleural SAA has a better diagnostic performance than CRP.

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Introduction

Pleural effusion is an accumulation of fluid in the pleural space that exceeds the physiological amount of 10–20 ml. Pleural effusion develops either when the formation of pleural fluid is excessive and/or when the fluid resorption is disturbed [1].

Diagnosis and management of pleural effusion remain a clinical challenge due to significant cost both to patients and to the health care system. In everyday clinical practice a variety of laboratory tests are used for the differential diagnosis of pleural effusions; however, a significant proportion remains undiagnosed [2].

A major problem remains the differentiation between malignant and benign effusions, as they have different outcome and management. Cytological examination of pleural fluid is a convenient and relatively efficient method for establishing the diagnosis of pleural malignancy. However, pleural fluid cytology is positive in only 50% of cases [3]; therefore, there is an increasing demand for markers that may help in this differentiation.

Serum amyloid A (SAA) and C-reactive protein (CRP) are acute-phase proteins predominantly produced and secreted by hepatocytes [4]. Other cells including lymphocytes, monocytes, and macrophages can also produce these proteins. The induction of SAA and CRP synthesis is triggered by a number of cytokines, chiefly IL-6, which is released from a variety of cell types, but mainly from macrophages and monocytes at inflammatory sites [4].

Increased serum CRP and SAA levels have been found in a number of pulmonary disorders, including bacterial infections, malignancies, and pulmonary thromboembolism. However, only a few studies have focused on their role in pleural effusions [5].

Aim

The aim of this study was to assess the diagnostic value of C-reactive protein (CRP) and serum amyloid A (SAA) in distinguishing different etiologies of pleural effusion and if they could discriminate between malignant and non-malignant pleural effusions.

Patients and methods

Study subjects

We investigated 92 patients with pleural effusion. Patients were divided into 2 groups:

Group I: (malignant pleural effusion), included 44 patients, [with male to female ratio (M/F)of 23/21 and a mean age of 57.7 ± 11.5 years in the form of mean ± 2SD].

Group II: (non- malignant or benign pleural effusion), included 48 patients, [with M/F ratio 33/15 and a mean age of 54.7 ± 10.4 years in the form of mean ± 2SD]. This group included tuberculous, parapneumonic and transudative pleural effusions.

Study design

Patients were subjected to:

1. History taking.
2. Clinical examination.
3. Chest radiography.
4. Thoracic ultrasound.
5. Tuberculin skin test: using 5 units P.P.D in 0.1 ml intradermal injection.
6. Laboratory investigations including: serum protein, LDH, liver and kidney function tests.
7. Aspiration of pleural fluid was done and was sent immediately for the following:
   a. Biochemical examination including: protein, LDH, C-reactive protein and serum amyloid A (SAA) levels.
   b. Cytological examination.
   c. Bacteriologic examination: Gram-staining, Ziehl–Neelsen stain and culture.
8. Tissue biopsy: One of the following was done according to case:
   a. Abram's needle pleural biopsy.
   b. Thoracoscopic biopsy; if the closed pleural biopsy is non diagnostic.

- Classification of pleural fluid into transudative or exudative is based upon Light's criteria (2002) [6]
- Transudative pleural effusion fulfills the following criteria:
  (1) Total fluid protein is less than half of that of the total serum protein level.
  (2) Fluid Lactate Dehydrogenase (LDH) is less than 0.6 of that of the serum LDH.
  (3) Pleural fluid LDH is less than two thirds the upper limit of the normal of that of the serum level.

- Effusions were considered malignant if malignant cells were found on the cytology examination of pleural fluid or in the pleural biopsy specimens,
- The diagnosis of tuberculous pleurisy was based upon high tuberculin positivity, lymphocytic pleural fluid, few mesothelial cells, elevated ADA level in the pleural fluid or pleural biopsy showing caseating granuloma.
- Criteria for parapneumonic effusion were; clinical, biochemical and radiological signs of suspected pneumonia, positive Gram staining, positive culture for bacteria or neutrophil predominance in pleural effusion.

Methods

*Serum and pleural SAA concentrations were determined using Enzyme-linked immunosorbent assay (ELISA) kit supplied by Assaypro (3400 Harry S Truman BlvdSt. Charles, MO 63301USA).

*Serum and pleural CRP concentrations were determined using ELISA kit supplied by ChemuxBioScience (South San Francisco, CA 94080, USA).

Statistical analysis

All statistical analyses were performed using the SPSS (Statistical Package for Social Sciences) version 17.0 software. Data were first tested by Kolmogorov–Smimov test for distribution of data. Data were expressed as mean and standard deviation (SD) for numerical parametric data. The mean and SD of the differences and the limits of agreement, defined as the mean ± 2 SD of the difference (95% CI), were calculated. ANOVA test was used for intergroup comparisons of means, A *P*-value of less than 0.05 indicated statistical significance. Correlations between numerical data were determined with the Pearson's rank correlation coefficient. Analysis of receiver operator characteristics (ROC) and calculation of the area under the curve (AUC) were used for both CRP and SAA in the pleural fluid.

Results

Of the 92 patients included in our study; 44 were diagnosed with malignant pleural effusion (group I) with male to female ratio (M/F) 23/21and 48 were diagnosed with non-malignant pleural effusion (group II) with an M/F ratio of 33/15. In the malignant group the mean ± SD of the age was 57.7 ± 11.5 years while in the non-malignant group it was 54.7 ± 10.4 years. Etiology of pleural effusion and demographic data of studied patients are presented in Table 1.

Levels of CRP and SAA in the pleural fluid and serum of the studied subjects are presented in Table 2 and Fig. 1. There were statistically significant differences regarding CRP, SAA and pleural/serum ratio of both markers among the studied groups ($P = 0.000$).

When Post Hoc Test, Least Significant Difference (LSD) was performed; pleural CRP was significantly higher in the parapneumonic group than transudate and tuberculous groups ($P < 0.05$), but no statistical significant difference was found between tuberculous and malignant groups ($P = 0.08$) or malignant and parapneumonic groups ($P = 0.25$). Moreover; pleural SAA values were significantly higher in both tuberculous and malignant groups than transudate and parapneumonic groups ($P < 0.05$), while there was no statistical significant difference between tuberculous and malignant groups ($P = 0.13$).

CRP and SAA values were significantly higher in both serum and pleural effusion of malignant vs. non-malignant group ($P < 0.003$) (Table 3), but there was no statistical significant difference as regards pleural/serum CRP and

Table 1 Etiology of pleural effusion and demographic data of studied patients.

Etiology of pleural effusion	Number of cases	Age (Mean ± SD)	M/F
Malignant (group I):			
(a) Lung cancer	6		
(b) Mesothelioma	2		
(c) Breast cancer	13		
(d) Renal carcinoma	26		
(e) Hepato-cellular carcinoma	6		
(f) Non-Hodgkin lymphoma	2	57.7 ± 11.5	23/21
(g) Cancer colon	4		
(h) Splenic cancer	2		
(i) Thyroid carcinoma	3		
(j) Cancer prostate	4		
Non-malignant (group II):			
(a) Tuberculous	12	51.3 ± 11	9/3
(b) Parapneumonic	19	55.6 ± 11	12/7
(c) Transudate:	17	56.2 ± 8.8	12/5
(liver- cell failure)	(9)		
(heart failure)	(8)		

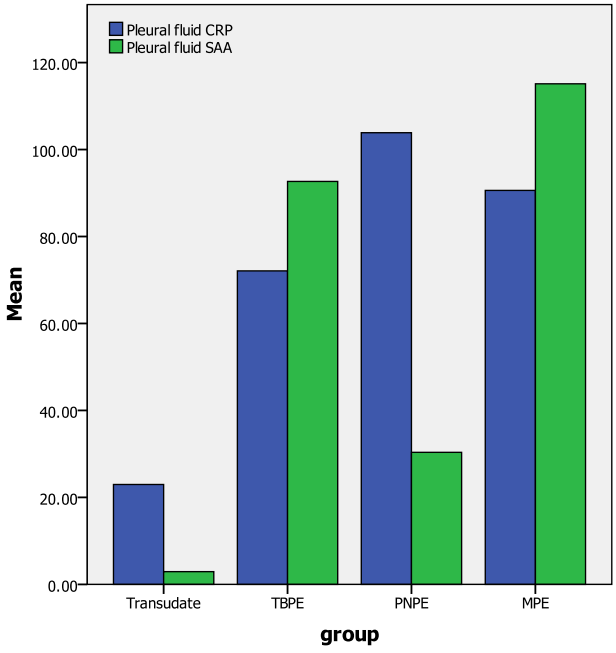

Figure 1 Levels of CRP and SAA in pleural fluid and serum of the studied subjects.

Table 2 Serum and Pleural CRP and SAA values in the different studied groups.

	Transudates ($n = 17$)	Parapneumonic ($n = 19$)	Tuberculous ($n = 12$)	Malignant ($n = 44$)	*P*
(a) Pleural hs-CRP (μg/ml)	23 ± 24.9	103.9 ± 28	72 ± 38	90.6 ± 34	0.000
(b) Serum hs-CRP (μg/ml)	67.6 ± 43.5	138 ± 27	116 ± 33	119 ± 27	0.000
(c) Pleural/serum hs-CRP	0.3 ± 0.2	0.8 ± 0.2	0.6 ± 0.2	0.8 ± 0.3	0.000
(a) Pleural SAA (μg/ml)	2.9 ± 1.3	30.4 ± 17.3	93 ± 37	115 ± 60	0.000
(b) Serum SAA (μg/ml)	40.9 ± 14.8	128 ± 35	206 ± 72	312 ± 114.4	0.000
(c) Pleural/serum SAA	0.08 ± 0.04	0.2 ± 0.1	0.5 ± 0.1	0.4 ± 0.2	0.000

pleural/serum SAA ratios between the two groups ($P = 0.148$ and $P = 0.453$ respectively).

The correlation analysis of pleural effusion for SAA, CRP (Fig. 2) using Spearman's test showed, in both malignant and non-malignant effusions, a statistically significant positive correlation between pleural fluid CRP and SAA ($r = 0.315$ and $P = 0.002$).

Receiver Operating Characteristic (ROC) curve for pleural fluid CRP and SAA in both infectious (including parapneumonic and tuberculous pleural effusions) and malignant pleural effusions was plotted Fig. 3.

Assessment of the diagnostic performance of pleural fluid CRP in both infectious and malignant effusions showed that at a cutoff value of 96.15 μg/ml for CRP; diagnostic sensitivity was 61% and specificity was 45%, while for pleural fluid SAA, a cutoff value of 137.5 μg/ml was associated with 41% sensitivity and 93% specificity. ROC curve shows that the AUC was higher for pleural fluid SAA than CRP (0.796 and 0.479, respectively).

Table 3 Serum and pleural CRP and SAA values in malignant and nonmalignant cases.

	Non-malignant ($n = 48$)	Malignant ($n = 44$)	P
Pleural CRP (μg/ml)	67.3 ± 46	90.6 ± 34	0.000
Serum CRP (μg/ml)	107.5 ± 46.4	119 ± 27	0.002
Pleural/serum CRP	±0.3	0.8 ± 0.3	0.148
Pleural SAA (μg/ml)	36.2 ± 41	115 ± 60	0.001
Serum SAA (μg/ml)	116.8 ± 77	312 ± 114.4	0.003
Pleural/serum SAA	±0.2	0.4 ± 0.2	0.453

Discussion

Pleural effusion (PE) is often a clinical problem in medical practice, as the differential diagnosis includes a wide variety of local and systemic diseases. Considerable effort has been made to develop a simple, inexpensive and noninvasive

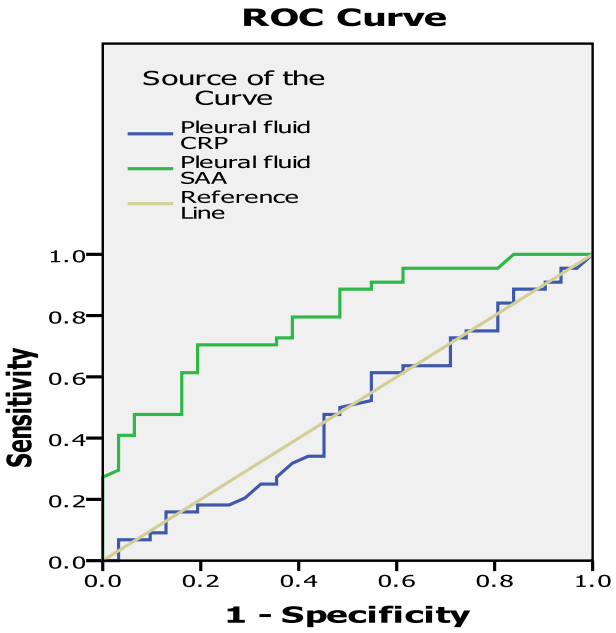

Figure 3 (ROC) curve for pleural fluid CRP and SAA in both infectious and malignant pleural effusions.

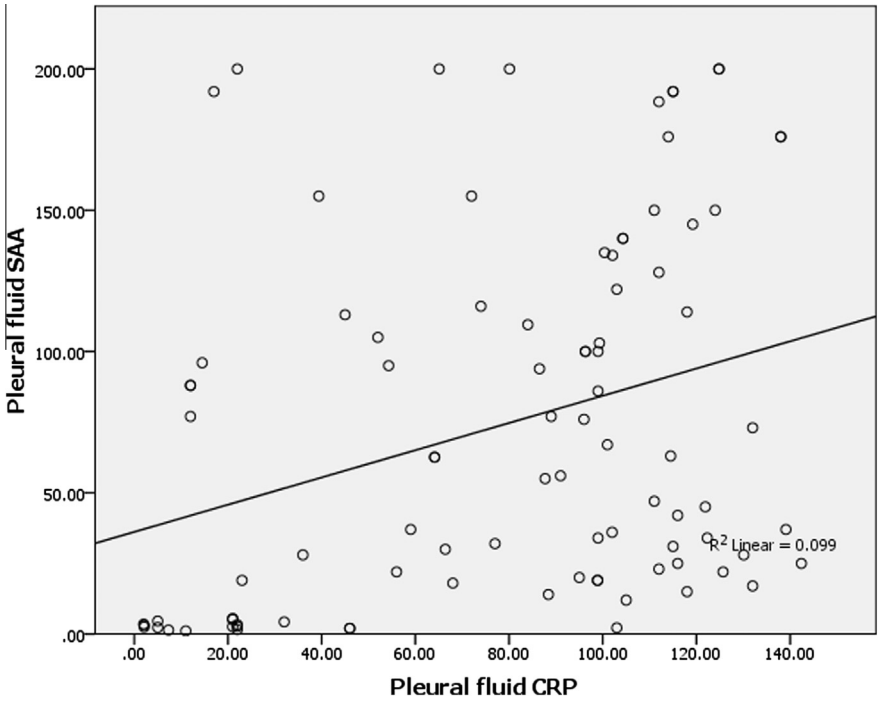

Figure 2 Correlation between pleural fluid CRP and SAA in malignant and non-malignant effusions.

method for distinguishing different types of PE in laboratory and clinical settings. No standard biochemical approach has yet been established. Ideal biomarkers should be easily measured at a reasonable cost (analytical validity), sensitive and specific to the disease state being examined, and aid in decision-making (clinical usefulness) [7].

SAA and CRP are acute-phase proteins predominantly produced and secreted by hepatocytes [4]. Although several studies have investigated the serum levels of the acute-phase proteins CRP and SAA in diseases, few have focused on the levels of these types of proteins in effusions.

Vidriales and Antaquera [8], Turay et al. [9], Tatjana et al. [10] found that pleural fluid CRP levels were highly elevated in parapneumonic effusion, than in other types of effusion.

Tatjana et al. [10] also found that there is no significant difference between malignant and tuberculous effusions as regards pleural fluid CRP levels. Our study showed similar results; pleural fluid CRP was significantly higher in the parapneumonic group than transudate and tuberculous groups ($P < 0.05$), but no statistically significant difference was detected between tuberculous and malignant groups ($P = 0.08$) or malignant and parapneumonic groups ($P = 0.25$). On the contrary, Chierakul et al. [11] and Garcia Patchon et al. [12] found that pleural fluid CRP levels were twice as high in tuberculous than in malignant effusion, while Turay et al. [9] found higher CRP effusion value in malignant effusion.

In agreement of our results, Hoda Abu-Youssef et al. [13] reported that there was a statistically highly significant difference for mean values of CRP between transudative and exudative pleural fluid effusions ($P < 0.003$) with higher levels in exudative effusion than those of transudative effusion.

An additional finding of our study is that pleural fluid CRP levels were higher in parapneumonic compared to tuberculous and malignant effusions. This may be attributed to the fact that CRP plays an important role in inflammation, as it increases profoundly in the region of inflammation [4].

Our study revealed that, pleural SAA values were significantly higher in both tuberculous and malignant groups than transudate and parapneumonic groups ($P < 0.05$), while there was no statistically significant difference between tuberculous and malignant groups ($P = 0.13$).

Our study demonstrated a statistically significant positive correlation between pleural fluid CRP and SAA in malignant and non- malignant effusions. In a research done by Alesssandra et al. [14] correlation analysis of serum and effusion SAA and CRP, showed a stronger correlation for SAA than for CRP in exudates and although SAA and CRP were highly correlated in the serum, they were only slightly correlated in exudates.

Assessment of the diagnostic performance of pleural fluid CRP and pleural fluid SAA in both infectious (including parapneumonic and tuberculous pleural effusions) and malignant pleural effusions showed that at a cutoff value of 96.15 μg/ml for CRP; diagnostic sensitivity was 61% and specificity was 45%, while for pleural fluid SAA, a cutoff value of 137.5 μg/ml was associated with 41% sensitivity and 93% specificity. ROC curve shows that the AUC was higher for pleural fluid SAA than CRP (0.796 and 0.479, respectively). A previous study done by Kiropoulos et al. [15] found that pleural fluid CRP was higher in parapneumonic compared to tuberculous and malignant effusions, providing 100% sensitivity and 79% specificity using a cut-off point of 5.3 mg/dL.

The study of Rezaeetalab and his colleagues [16] reported that in discrimination between the exudates and transudates, a cutoff value of 5 mg/L for pleural fluid CRP showed 94% sensitivity and 96.6% specificity.

In summary, in this prospective study of acute phase markers in pleural effusions of different etiologies; we reported that at a cutoff value of 96.15 μg/ml for CRP; and 137.5 μg/ml for SAA in pleural fluid; these two markers may have diagnostic utility for the differentiation of infectious (parapneumonic and tuberculous) effusion from malignant effusions and pleural SAA has a better diagnostic performance than CRP. CRP and SAA determinations are relatively simple, rapid, and inexpensive and in this study we find that they may contribute to this discrimination.

Conclusion

Measurement of SAA and CRP levels in pleural fluid has a good diagnostic utility in differentiation between malignant and non-malignant pleural effusion and pleural SAA has a better diagnostic performance than CRP.

Conflict of interests

The authors declare that there is no conflict of interests.

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